March 2019

Apple Disease Forecasting Models: When Climate Changes the Rules

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A Thesis Presented

by

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Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

MASTER OF SCIENCE

February 2019

Plant and Soil Sciences

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ACKNOWLEDGMENTS

I would like to thank my advisor and committee chair Dr. Daniel Cooley and mentor Mr. Jon Clements for their patient guidance while I navigated the treacherous waters of life as a non-traditional student. I would also like to thank my committee members Dr. Wesley Autio, Dr. Robert Wick and Dr. Terence Bradshaw, without whom this thesis would not be possible. I owe a debt of gratitude to Lyndsey Ware, Cam Olanyk, Paul O'Connor, and Nicole Foley for counting what surely must amount to hundreds of thousands *Venturia inaequalis* ascospores. I would especially like to thank Arthur Tuttle for being the first to introduce me to the joys and woes of *Venturia inaequalis* microscopy.

I am thankful to Ben and Tom Clark of Clarkdale Fruit Farm, Al Rose of Red Apple Farm, Zeke Goodband of Scott Farm and John Rogers of Rogers Orchard for allowing me to use their sites for portions of this research. I would also like to thank Amanda Brown for lending me space at the UMass Agricultural Learning Center and Shawn McIntire, Jim Krupa and Jim Cronk of the UMass Cold Spring Research Orchard for always looking out for the welfare of my research there. I am thankful for funding provided by USDA NIFA Extension Implementation Program, grant no. 2-14-700006-22579/project accession no. 1005350, and Integrated Pest Information Platform for Extension and Education award number 2015-68004-23179.

More than all these and more than any words can express, I am deeply grateful to my husband, Mike Ekblom and our children Holden, Isabella, and Delia for forgiving the late nights, missed conferences and occasional bad moods and for cheering me on every step of the way. I would also like to thank my mother Mary Fiske for covering the aforementioned missed meals (and bus pick ups, and guitar lessons…).

ABSTRACT

APPLE DISEASE FORECASTING MODELS: WHEN CLIMATE CHANGES THE RULES

FEBRUARY 2019

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With a changing global climate, plant pathologists must understand the impact aberrant weather events may have on the development of plant diseases. Fungal plant infections are largely dependent on temperature and precipitation, climate parameters that are predicted to change more in this century. *Venturia inaequalis* causes apple scab, one of the most destructive apple diseases of temperate growing regions. Temperature and precipitation drive apple scab infections and forecast models, which guide growers in efficient, effective fungicide applications. In some recent years in the Northeast, these models have failed to accurately predict when ascospores of this fungus are available to cause primary infections, prompting more fungicide intensive management. Identifying cause(s) of model failures will restore confidence in them, enabling growers to reduce fungicide use. As technology becomes an increasingly important component of on farm decision-making, so does educating new farmers and agricultural students in the benefits of Integrated Pest Management and challenges associated with models early on in their college educational experience. This research attempts to identify reasons for ascospore maturity model failures, determine to what

degree critical ascospore maturity parameters have changed and create a tool that educators may use to engage undergraduate students in the complexities of Integrated Pest Management research and modern farming. It will more specifically do the following: 1) Dry periods will be analyzed to determine if frequency and duration are increasing, causing the fungus to mature over a longer period of time than models currently estimate. 2) Degree-days during fall and winter will be examined to estimate what effect a warming climate may have on ascospore and tree development, and ultimately apple scab occurrence. The research will use lab and field observations to track the development of *V. inaequalis* ascospores, the source of primary apple scab infections. These observations will be compared to infection events and spore maturation forecasts from models currently used by apple growers in the Northeast. 3) A case study developed for publication in American Phytopathological Societies' Plant Health Instructor will provide early career college students with an introduction to forecasting models, Integrated Pest Management and the challenges associated with climate variability.

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ASSESSING DECISION SUPPORT SYSTEM ACCURACY IN DETERMINATION OF *VENTURIA INAEQUALIS* ASCOSPORE MATURITY AND PRIMARY INFECTIONS

1.1 Abstract

Accurate prediction of potential for infection and inoculum availability is critical to preventing infections during the primary period for apple scab, a disease caused by the fungus *Venturia inaequalis* (Cke) Wint. The fungus overwinters in infected apple leaves on the orchard floor. Pseudothecia, the fungal fruiting bodies, develop in the leaves. In the spring, ascospores are released form pseudothecia during a rain event. Given the right conditions, these ascospores can cause primary apple scab infections. Infection and ascospore maturity models are included in DSSs used by growers in the Northeast. Confidence in a DSSs ability to adequately evaluate these events is necessary if they are to be employed in IPM programs. This study compared ascospore maturity and infection events observed in the field and laboratory to those estimated by four commonly used DSSs. From 2016 to 2018 nine site-year combinations were observed in MA and CT. For all site-year combinations, mature ascospores were observed in lab assays prior to maturity initiation estimated by DSSs. Mature spores were observed after 100% maturity was estimated by some DSSs in all three years. In addition, infection events were monitored in situ using potted trees. In each site/year, the number of primary infection event periods observed in trees differed from that estimated by DSSs.

1.2 Introduction

Venturia inaequalis (Cke) Wint., the fungal pathogen that causes the disease apple scab on *Malus* species, has plagued apple growers since orchards shifted from sparsely planted trees across large tracts of land to the more densely planted agroecosystems many have come to know across New England today (MacHardy et. al. 2001). The fungus has a long evolutionary history with its host, and has moved around the world with the domesticated apple (Gladieux et al., 2008; Le Van et al., 2012).

To aid growers in management of this pathogen, models that estimate development and release of the primary inoculum (Gadoury & MacHardy 1982), and its subsequent infection of the host have been created and deployed (Holb, 2006; Mills, 1944). Ascospores, the result of sexual reproduction of the fungus, are the cause of these primary infections. Continued verification of the accuracy of predictive models used in disease management and Integrated Pest Management (IPM) programs has been identified as critical to ensuring Decision Support Systems (DSSs) containing these models remain a valid method of advising growers in effective fungicide use decisions (MacHardy & Gadoury, 1985). This is especially true for climates that are drier than that in which current models were developed (MacHardy et al., 2001).

Accuracy is particularly important in estimates of initiation and completion of ascospore maturation, and ejection of the last ascospores in a given year, as these define the beginning and end of the period when primary apple scab infections are possible. If inaccurate, late season infection events may be missed and early season events estimated that do not occur. These inaccuracies have the potential to cause unnecessary fungicide use or cause a fungicide application to be missed and infections established. Any primary infection event has the potential to lead to a season-long battle with disease and reduced fruit quality and yield.

The equipment and training necessary for accurate ascospore maturity assessments are expensive and time-consuming. This inspired the development of the ascospore maturity model (Gadoury et al., 2004). To streamline the process of estimating primary inoculum availability, plant pathologists have developed models, based on environmental parameters, particularly temperature, to estimate the development of *V. inaequalis* ascospores. Collecting temperature data is much easier than direct observation of pseudothecia and ascospores, and gives growers on-site information about ascospore availability, and infection potential during the growing season. Heat unit accumulation above a given base temperature, which varies by organism, is used to estimate the effect of temperature on many biological processes, including fungal development. These heat units are referred to as degree-days (DD) (Allen, 1976; Baskerville & Emin, 1969). The base temperature used for *V. inaequalis* ascospore development is 32°F. Below this temperature, little or no biological activity occurs (James & Sutton, 1982a; MacHardy & Gadoury, 1985). These models also require a starting time that is used as the date on which to begin recording temperatures or any other required environmental data. Gadoury and MacHardy (1982) developed a temperature-driven model, hereafter referred to as the New Hampshire or NH model. This model tracks ascospore maturation from the time the first mature spores are available to the point at which they have all matured and been ejected. The NH model used the first observed mature ascospores as a biofix, and a base of 0° C (32° F), in a temperature-driven linear model. This model was subsequently updated, substituting the host phenophase 'silver tip' as a biofix (MacHardy & Gadoury, 1985). In later applications of the model, green-tip (Fig. 1) has been used (e.g. Network for Environment and Weather Applications, Cornell IPM, http://newa.cornell.edu). The basic approach, using DDs to estimate the period of possible primary apple scab infections, has been widely adopted around the world, though the model parameters vary somewhat (Alves & Beresford, 2013; Rossi et al., 1999; Roubal & Nicot, 2016).

Once the period of potential primary infections is known, growers need to know whether conditions that cause an infection have occurred or are likely to occur. W.D.

Mills (1944) developed the primary infection model for apple scab on which many current models are based. Given inoculum in the orchard, hours of wetting and concurrent average temperatures are used to calculate whether or not there would be an infection event, and its severity. When leaves are wet, infection at warmer temperatures requires less time than at colder temperatures. For example, according to the Mills' Table, at 60° F 20 hours of continuous leaf wetting will cause a severe scab infection. The original Mills model does not take into consideration other factors that contribute to spore fitness and the infection process. Noting discrepancies between the Mills Table and later observations made by other researchers, MacHardy and Gadoury (1989) modified the criteria to account for daylight spore release. Stensvand and colleagues (1997) further revised the Mills Table, reducing the time required for infection at low temperatures.

Jones et al. (1984; 1980) made the first on-farm computer integrated with weather sensors in a system that evaluated apple scab infections. The microcomputer system more accurately measured temperature, relative humidity and moisture, and automated the process of infection period determination. These were only available in the field. The most recent applications of disease forecast models to plant disease management are in the form of online DSSs. These systems use weather data, which is fed into web-based platforms, containing models, such as the two models for apple scab. Users provide a limited number of field observations, such as host phenophases. The DSS combines this information using algorithms and decision rules and computes risk, in this case, the risk of an apple scab infection. This risk is then communicated via a user interface.

There are four DSSs commonly used in the Northeast for predicting apple scab risk: Ag-Radar, developed by Glen Koehler, University of Maine Cooperative Extension; SkyBit, developed by Joseph Russo as part of ZedX, Inc. (Bellefonte, PA) owned by

BASF Group; RIMpro, developed by Marc Trapman, (Bio Fruit Advis, Netherlands); and the Network for Environment and Weather Applications (NEWA), a collaboration of Cornell University and the Northeast Regional Climate Center (Table 1.1). The first two use virtual weather data to predict, among other pests, the potential for apple scab based on inoculum availability and environmental parameters, particularly rain periods and temperature. RIMpro uses either virtual data (Yr, Norway) or AWS data. SkyBit uses virtual data generated using its proprietary system. Ag-Radar also uses SkyBit's virtual weather data. NEWA uses AWS data from stations at orchards, or National Weather Service data from airport weather stations.

These four DSSs incorporate a model basically comprised of two components, an ascospore maturity model and an infection period model (Fig. 2). However, the structure and complexity of the models varies by DSS. For example, RIMpro and Ag-Radar account for spore death due to leaf litter decomposition and leaf wetness duration inadequate to promote infection (Philion et al., 2009).

All DSSs require a biofix to begin the ascospore maturity model, though there are differences in what is used and how it is entered into the system. NEWA uses the green-tip phenophase of 'McIntosh', either estimated by the system or, if inaccurate, input by the user. SkyBit requires customers to report a green-tip biofix then input by the company. Similarly, Ag-Radar requires users to report a green-tip date input by the service. RIMpro has four options for setting the biofix, in a hierarchical, preferential order: 1) first ascospores discharged in the lab via Petri-plate assay; 2) first spores observed in the field using spore traps; 3) first mature spores observed in squash mount OR green-tip (GT) of main scab susceptible cultivar on site. In the northeast United States, 'McIntosh' is used to determine GT.

The intent of this study was to analyze the accuracy of ascospore maturity and infection estimates for apple scab of the four different DSSs, comparing them to observed ascospore maturity and infections.

1.3 Materials and Methods

1.3.1 Study Sites

Field validation of infection events and field and lab observation of ascospore maturity were studied at a number of sites in southern New England from 2016 through 2018. In 2016, ascospore maturity and infection events were observed for two sites in MA: the University of Massachusetts' Cold Spring Orchard (CSO) in Belchertown; and Clarkdale Fruit Farms (CFF) in Deerfield. In 2017, an additional MA site was added, the University of Massachusetts' Agricultural Learning Center (ALC), in Amherst. Ascospore maturity and infection events were observed at all locations in 2017. In 2018, one additional site was added, Roger's Orchard (RGO) in Southington, CT. Ascospore maturity and infection events were observed at all sites for 2018 using both field and lab assays and four DSSs. In order to standardize the biofix across DSSs, green-tip was used for each site/year combination.

These locations are research, teaching or commercial apple orchards, each equipped with an automated weather station (AWS; Rainwise Inc. Trenton, ME; or Onset Computer Corporation, Bourne, MA), which provide real-time weather data from each orchard. While some of these locations are relatively close geographically, important environmental variables can differ significantly between each one, including DD accumulation and timing of precipitation and drying periods (Magarey et al., 2001). The AWSs record data and sent it to DSS servers. Alternatively, and in some cases additionally, weather data was supplied to DSSs by virtual weather data services (Yr, Norwegian Meteorological Institute and Norwegian Broadcasting Company, Norway; or SkyBit, Inc., Bellefonte, PA).

1.3.2 Ascospore Maturity

In the fall of 2015, 2016 and 2017 scab-infected leaves were collected from unsprayed trees, before they fell, and overwintered in an outdoor location at study sites (CSO and CFF in 2015-16; CSO, ALC and CFF in 2016-17; CSO, CFF and ALC in 2017-18). The exception was RGO in 2018, where leaf samples were transported from CSO to RGO on 3 Apr 2018, which may have had an effect on maturity data, as the pathogen did not overwinter at RGO.

At each site, leaves were placed on the ground in a location as closely resembling that found in orchards as feasible, without exposing commercial orchards to greater infection risk, or the leaves to fungicide applications. In order to prevent leaf decay, a layer of landscape fabric was laid on the ground, and on this the scab-infected apple leaves were spread out, adaxial surface up, in a single layer. Those were then covered with a layer of hardware cloth to prevent loss from wind or animal activity. This also allowed the negatively geotropic pseudothecia to develop uniformly (i.e. ostiole facing upward).

From each location, once per week, six leaves were collected and brought to the lab for analysis of ascospore maturity (ASM). ASM was assessed using laboratory Petri-plate assays (PPA) of spore discharge as described by Szkolnik (1969) and Gadoury et al. (2004). Leaves were soaked in deionized water at room temperature for five minutes to induce spore release, and then placed into the bottom portion of a Petri plate that had been prepared with double-sided tape. Two microscope slides were placed in the top portion of the Petri plate (Fig. 3). The bottom was then placed on top so that the Petri plate was upside down with the infected leaf surface facing the slides below. After one hour the slides were examined for the presence of spore, by checking ten random fields on each slide at 200X magnification. Numbers of spores were recorded.

1.3.3 Infection Periods

DSS accuracy was analyzed using "trap trees" (Philion et al., 2009; Trapman, 1994). This field test was used to establish when apple scab infections actually occurred in an orchard during a putative infection event estimated using the DSSs.

The infection periods estimated by the DSSs varied by DSS. RIMpro and Ag-Radar give measures of the relative severity of each infection, while NEWA and SkyBit provide only yes or no information. Trap trees were used to determine if a given rain event caused infection.

In RIMpro, a Relative Infection Measure (RIM) represents the occurrence and severity of infection events. Events with a RIM of 100 or greater are considered for this analysis. This value is based on a virtual "bank" of 10,000 spores presumed to be available within the framework of the RIMpro model (Philion et al., 2009; Trapman, 1994). Infection output from Ag-Radar is measured in terms of the total seasonal infection potential. This potential is based on a percent scale represented by the total seasonal ascospore maturity. It assumes that, given inoculum in the orchard, spore availability is relatively low at the beginning of the season and when spores begin to mature. Potential increases exponentially until peak maturity and release occur, at which point, the potential for infection once more begins to decline as spore availability decreases. NEWA and Skybit track the same ASM curve, as it relates to infection, but do not represent infection potential with the complexity that RIMpro and Ag-Radar do. Both RIMpro and Ag-Radar present the user with either table or graphical output, which includes risk severity. NEWA tells users whether an infection has occurred on a day-by-day basis using tabular, yes/no format. Similarly, SkyBit indicates whether or not an apple scab infection has occurred on a given day using a table.

Prior to each forecast rain event during the period when primary infections might occur, four potted apple trees, cv. 'McIntosh', rootstock G.41, were removed from the nursery site, a hoop house at CSO in Belchertown MA, and transported to each test site. The trees were placed around a collection of scab-infected apple leaves, described above. After each rain event, once leaves on the potted trees had dried, trees were removed from the sites and returned to the hoop house at CSO, where they were protected from further wetting and thus infections, and observed for the development of disease signs and symptoms. Exposed trees were separated within the hoop house to prevent spores from primary lesions being transferred to unexposed trees. New, unexposed trees were placed at each site when the potentially infected trees were removed. By placing uninfected trees on site prior to each forecast rain event during the primary infection season, we are able to determine if the infection event estimated by each DSS associated with a given rain event did or did not occur. To assess the presence of infection, all leaves on each tree are monitored for infection. If lesion(s) were observed on any leaf, the rain event was determined to have been an infection event.

1.4 RESULTS

1.4.1 Ascospore Maturity

- 2016 (Table 1.2)

Green-tip (GT) was recorded at CSO on 31 Mar, Ag-Radar, NEWA and RIMpro estimated the first mature ascospores that day, while the SkyBit estimate was over two weeks later, 15 Apr. Ascospores were observed in the Petri-plate assay (PPA) on 28 Mar. All DSSs except RIMpro estimated 100% ASM at the end of May, well before the PPA date of 21 Jun. RIMpro estimated 100% ASM three days after the PPA, 24 Jun.

GT occurred at CFF on 30 Mar. Ag-Radar and NEWA initiated ascospore maturity on that date, RIMpro on 31 Mar, while SkyBit delayed initiation estimations until 15 Apr. First spores were observed in the PPA one day prior to GT. As with CSO, all DSSs except RIMpro estimated 100% ASM nearly a month prior to PPA observations, with the RIMpro estimate being only three days before the PPA date.

- 2017 (Table 1.3)

GT was observed on 10 Apr at CSO, ALC and CFF. Ag-Radar, NEWA and RIMpro all estimated the first mature spore for all three sites within two days of this date, while for all sites SkyBit reported first ascospores approximately one week later. Spores were first observed after GT in the PPA for all sites, only two days later at CSO and ALC but three weeks later at CFF. 100% ASM was estimated from two weeks to two days earlier than the final observed ascospores in PPAs at all sites by all DSS except RIMpro, which estimated 100% maturity from 18 to 24 days later, depending on the site.

- 2018 (Table 1.4)

CSO, CFF and ALC reached GT on 18 Apr, and RGO on 14 Apr. Ag-Radar, NEWA and RIMpro had very similar dates for the start of ASM, 18 to 19 Apr at CFF, ALC and CSO, and 14 Apr at RGO. SkyBit estimated the first ascospore availability 9 to 11 days later. At all sites 100% ASM was estimated by all DSSs except RIMpro prior to the final observed PPA spore release. RIMpro estimated 100% ASM as significantly later than PPA observations, from two and a half to over five weeks later.

1.4.2 Infection Periods

- 2016 (Table 1.5)

At CSO, DSSs estimated the first infection event to have occurred earlier than that observed in trap trees, and estimated more events to have occurred than were observed on trap trees. RIMpro estimated one event less than the number observed. NEWA and Skybit estimated the final infection event to occur five days prior to the final event as observed on trap trees, whereas Ag-Radar and RIMpro estimated the final event to occur after that observed.

At CFF, four infections were observed on trap trees. All DSSs estimated the first event to have occurred prior to those observed on trap trees as well as estimating more events to have occurred. RIMpro and Ag-Radar estimated the final event to occur after that observed on trap trees and NEWA and Skybit both estimated the final event to occur on the same day as observed in trap trees.

- 2017 (Table 1.6)

Ag-Radar estimated more infection events at each site than were observed in the trap trees. At CSO NEWA, RIMpro and Skybit estimated the same number of infections as were observed in trap trees. At CFF and ALC there were six infection events observed in trap trees, the number reported by each DSS varied by site. DSSs estimated infection events to occur prior to those observed on trap trees for all sites. At CSO, DSS estimated the final infection event to occur after that observed on trap tress. At CFF Ag-Radar, RIMpro and Skybit estimated the final infection event to occur after that observed on trap trees, NEWA before. At ALC, Ag-Radar and Skybit estimated the final event to have occurred 2 days prior to that observed on trap tress while NEWA and RIMpro estimated the final event to occur after that observed on trap trees.

- 2018 (Table 1.7)

All DSS estimated more infection events to occur at all sites than were observed in trap trees. At CSO all DSS estimated the first infection event to occur prior to that observed in trap trees, with the exception of RIMpro which estimated the first event to occur as the same day as observed on trap trees. Each DSS estimated the final infection event to occur on or after the date of the final event observed on trap trees except Skybit which estimated the final event to occur prior to that observed. At CFF, ALC and RGO, all DSS estimated infection events to occur prior to those observed in trap trees. Additionally, each DSS estimated the final event to occur at these locations after those observed on trap trees.

1.5 DISCUSSION

The ultimate test of the utility of a DSS in scab management is whether it can accurately estimate infections. Assuming accurate weather data, inaccurate estimates can come from errors in the models for ascospore maturity or infection.

The critical aspects of ascospore maturity estimates are when the first spores are mature enough to be released, and when all of the spores have matured and been released. Three of the DSSs use the green-tip phenophase to start the maturation model, while RIMpro encourages use of the first observed spores when that information is available. In this study, however, all DSS/site/year combinations used the green-tip date as a biofix in order to determine variability between DSS estimates for the end of ascospore maturation from a common start, and because growers generally do not make observations of ascospore release. Both Ag-Radar and RIMpro provide more detail and depth in their observations, and future DSS comparisons should determine whether this improves management as compared to the less detailed NEWA and Skybit systems.

For the purpose of validating ASM estimations from DSSs, the Petri-plate assay (PPA) as established by Szkolnik (1969) and Gadoury et al. (2004) was selected as an observational method best representing the temporal development of ascospores in infected leaf litter in the field. With one exception, for each site-year combination, each DSS estimated ASM to initiate on or after the GT date, while PPAs indicated spore maturation prior to GT. The exception was RGO, which, as previously stated, had leaves from Belchertown MA placed at the Southington CT site in the spring of 2018, probably disrupting the maturation process and confounding the PPA date. Additionally, in 2017, GT occurred prior to first observed spore releases. The discrepancies in ASM start dates relative to PPA assessments suggest that GT is not always an appropriate date to use for the accumulation of DD and other climatic factors contributing to ASM. That RIMpro's hierarchical list of biofix options gives preference to PPA spore observations supports that theory. Future steps for this work should include DSS evaluations of ASM using both GT and PPAs as biofix dates to determine whether this significantly changes ASM estimates later in primary season, particularly the 100% ASM date.

RIMpro was the only DSS that estimated ASM to continue beyond that observed in PPAs. According to Ficke et al. (2002), risk of primary scab infection over the phenological development of trees from green-tip to petal fall is related to three factors: inoculum availability, host tissue susceptibility and host tissue target area. Normally, risk is very low at GT, increases through bloom, and then drops to a low risk at petal fall and fruit set. However, if the pattern of ascospore development is disrupted such that there is relatively more inoculum available at petal fall than normally occurs, the risk of infection at the end of primary scab season will be relatively higher. If a DSS underestimates the amount of inoculum available at this time, particularly if it estimates all inoculum has matured and been released, and growers stop fungicide applications, it can result in scab infections. Of course, this will depend on whether and when infection events occur after a DSS estimates the end of primary scab season. As noted earlier, DSS estimates of primary scab infection depend on two models, one for ASM and the other for an infection period.

The potted trap trees (TTs) indicated when the potential for infection actually ended. Final infection dates represent the date that the rain event with an infection began, in the case of trap trees. DSS final infection dates represent the date that each DSS estimated infection potential to begin. This varies by DSS. RIMpro, for example does not necessarily assign a RIM value to an infection event at the onset of rain. Skybit, however, estimates rain to equate to an infection event with no severity information. In 2016, NEWA and Skybit both estimated an end to infection risk at CSO six days before TTs indicated the last infection. Had a grower been relying on these models, it may have caused a fungicide application to be skipped, leading to infection. All other site-DSS combinations for 2016 were sufficient to protect from infection. RIMpro, however, as a result of its extended ASM estimates, estimated an additional infection at CFF six days after the final infection event was observed, which could cause an unnecessary fungicide application for management of primary apple scab.

In 2017, at CSO, RIMpro and Ag-Radar estimated the final significant primary infection to begin six days prior to that observed in trap trees. Skybit and NEWA estimated the final infection event to begin nine days prior to those observed, potentially leading to infection due to cessation of fungicide applications for primary scab. At CFF, Ag-Radar and RIMpro estimated final events to begin after those observed, Skybit two days prior and NEWA more than two weeks prior. In this case, NEWA estimations would have led to insufficient fungicide coverage. At ALC, NEWA and RIMpro estimated final infection events after those observed on trap trees and Ag-Radar and Skybit two days prior.

In 2018, Skybit estimated an early end to the final primary infection event at CSO and ALC. All other DSS-site combinations for this year estimated final infections to initiate on the same date as observed in trap trees or estimated additional infection events to occur beyond those observed in trap trees. In two instances, Ag-Radar overestimated by 14 and 15 days (CFF and ALC), leading to potentially unnecessary primary scab fungicide use. Additionally, RIMpro overestimated the date of final primary infection by two weeks or more at all sites, even in a conservative fungicide program. This would lead to inefficient primary apple scab fungicidal applications.

While further work is necessary to pinpoint parameters affecting accuracies in DSS, we can confidently recommend RIMpro. There is the potential for additional fungicide applications resulting from conservative end of season ASM and infection events predicted, depending upon which biofix date is selected. However, given that apple wholesalers and grocery stores reject fruit with scab on it, a grower must err on the side of caution when managing this disease.

Table 1.1. Decision support systems used for apple scab in the northeastern US with weather information sources.

Decision Support System	Weather Information Source, Charge
Ag-Radar University of Maine Extension	ZedX, Inc. proprietary virtual data supplied via SkyBit subscription
NEWA Network for Environment and Weather Applications, NY IPM, Cornell University	Weather stations (must supply correctly formatted data). Fee for station may be required.
RIMpro BioFruit Advies, Zoelmand, Netherlands	Weather stations or virtual data via private companies. Charge for RIMpro and virtual data.
SkyBit ZedX, Inc. recently purchased by BASF	Proprietary virtual data, charge.

Table 1.2. Critical ascospore development dates estimated by decision support systems compared with green-tip and Petri-plate spore release observations in 2016 for two sites.

		First mature ascospore					100% ascospore maturity				
Site[a]	**GT**[b]	**Ag-R**[c]	**NEWA**	**RIMpro**	**SkyBit**	**PPA**[c]	**Ag-R**	**NEWA**	**RIMpro**	**SkyBit**	**PPA**
CSO	31 Mar	31 Mar	31 Mar	31 Mar	15 Apr	28 Mar	29 May	24 May	24 Jun	28 May	21 Jun
CFF	30 Mar	30 Mar	30 Mar	31 Mar	15 Apr	29 Mar	28 May	26 May	21 Jun	28 May	24 Jun

[a]CSO = UMass Cold Spring Research Orchard, Belchertown, MA; CFF= Clarkdale Fruit Farm, Deerfield, MA
[b]Green-tip phenological date
[c]Ag-R = Ag-Radar; PPA = Petri-plate assay

Table 1.3. Critical ascospore development dates estimated by decision support systems compared with green-tip and Petri-plate spore release observations in 2017 for three sites.

		First mature ascospore					100% ascospore maturity				
Site[a]	**GT[b]**	**Ag-R[c]**	**NEWA**	**RIMpro**	**SkyBit**	**PPA[c]**	**Ag-R**	**NEWA**	**RIMpro**	**SkyBit**	**PPA**
CSO	10 Apr	11 Apr	10 Apr	12 Apr	16 Apr	12 Apr	24 May	20 May	24 Jun	27 May	6 Jun
CFF	10 Apr	11 Apr	11 Apr	11 Apr	16 Apr	2 May	28 May	20 May	24 Jun	28 May	30 May
ALC	10 Apr	10 Apr	11 Apr	12 Apr	16 Apr	12 Apr	24 May	21 May	30 Jun	28 May	6 Jun

[a]CSO = UMass Cold Spring Orchard, Belchertown, MA; CFF= Clarkdale Fruit Farm, Deerfield, MA; ALC = UMass Ag. Learning Center, Amherst, MA.
[b]Green-tip phenological date
[c]Ag-R = Ag-Radar; PPA = Petri-plate assay

Table 1.4. Critical ascospore development dates estimated by decision support systems compared with green-tip and Petri-plate spore release observations in 2018 for four sites.

		First mature ascospore					100% ascospore maturity				
Site[a]	**GT[b]**	**Ag-R[c]**	**NEWA**	**RIMpro**	**SkyBit**	**PPA[c]**	**Ag-R**	**NEWA**	**RIMpro**	**SkyBit**	**PPA**
CSO	18 Apr	18 Apr	18 Apr	19 Apr	28 Apr	3 Apr	30 May	26 May	6 Jul	31 May	18 Jun
CFF	18 Apr	18 Apr	18 Apr	19 Apr	28 Apr	2 Apr	30 May	26 May	6 Jul	31 May	18 Jun
ALC	18 Apr	18 Apr	18 Apr	19 Apr	28 Apr	7 May	30 May	26 May	10 Jul	31 May	4 Jun
RGO	14 Apr	14 Apr	14 Apr	14 Apr	25 Apr	25 Apr	27 May	23 May	22 Jul	29 May	14 Jun

[a]CSO = UMass Cold Spring Orchard, Belchertown, MA; CFF= Clarkdale Fruit Farm, Deerfield, MA; ALC = UMass Ag. Learning Center, Amherst, MA; RGO = Roger’s Orchard, Southington, CT.
[b]Green-tip phenological date
[c]Ag-R = Ag-Radar; PPA = Petri-plate assay

Table 1.5. Comparison of infection periods determined by four decision support systems as compared to infections in potted trees, 2016.

	First infection period					Final infection period					Number of infection periods				
Site[a]	**Ag-R**[c]	**NEWA**	**RIMpro**	**SkyBit**	**TT**[b]	**Ag-R**[b]	**NEWA**	**RIMpro**	**SkyBit**	**TT**[b]	**Ag-R**[b]	**NEWA**	**RIMpro**	**SkyBit**	**TT**[b]
CSO	22 Apr	2 Apr	7 Apr	22 Apr	1 May	7 Jun	30 May	11 Jun	30 May	5 Jun	8	8	6	8	7
CFF	22 Apr	1 Apr	7 Apr	22 Apr	1 May	5 Jun	30 May	5 Jun	30 May	30 May	8	8	7	7	4

[a]CSO = UMass Cold Spring Orchard, Belchertown, MA; CFF= Clarkdale Fruit Farm, Deerfield, MA
[b]Ag-R = Ag-Radar; TT = trap trees, potted trees placed in orchards for each potential infection period.

Table 1.6. Comparison of infection periods determined by four decision support systems as compared to infections in potted trees, 2017.

	First infection period					Final infection period					Number of infection periods				
Site[a]	**Ag-R**[c]	**NEWA**	**RIMpro**	**SkyBit**	**TT**[b]	**Ag-R**[b]	**NEWA**	**RIMpro**	**SkyBit**	**TT**[b]	**Ag-R**[b]	**NEWA**	**RIMpro**	**SkyBit**	**TT**[b]
CSO	12 Apr	2 Apr	20 Apr	17 Apr	21 Apr	29 May	27 May	29 May	27 May	5 Jun	11	8	8	8	8
CFF	12 Apr	16 Apr	20 Apr	16 Apr	21 Apr	31 May	13 May	4 Jun	30 May	29 May	13	6	5	7	6
ALC	12 Apr	16 Apr	20 Apr	16 Apr	21 Apr	29 May	16 Jun	16 Jun	29 May	31 May	10	7	6	8	6

[a]CSO = UMass Cold Spring Orchard, Belchertown, MA; CFF= Clarkdale Fruit Farm, Deerfield, MA; ALC = UMass Ag. Learning Center, Amherst, MA.
[b]Ag-R = Ag-Radar; TT = trap trees, potted trees placed in orchards for each potential infection period.

Table 1.7. Comparison of infection periods determined by four decision support systems as compared to infections in potted trees, 2018.

	First infection period					Final infection period					Number of infection periods				
Site[a]	**Ag-R**[c]	**NEWA**	**RIMpro**	**SkyBit**	**TT**[b]	**Ag-R**[b]	**NEWA**	**RIMpro**	**SkyBit**	**TT**[b]	**Ag-R**[b]	**NEWA**	**RIMpro**	**SkyBit**	**TT**[b]
CSO	25 Apr	25 Apr	3 May	28 Apr	3 May	4 Jun	4 Jun	18 Jun	1 Jun	4 Jun	11	7	8	8	5
CFF	25 Apr	25 Apr	6 May	28 Apr	12 May	4 Jun	4 Jun	23 Jun	1 Jun	1 Jun	11	7	7	7	4
ALC	27 Apr	25 Apr	27 Apr	28 Apr	6 May	5 Jun	4 Jun	24 Jun	1 Jun	4 Jun	11	8	8	7	7
RGO	25 Apr	25 Apr	25 Apr	16 Apr	12 May	2 Jun	2 Jun	23 Jun	30 May	27 May	12	5	5	7	4

[a]CSO = UMass Cold Spring Orchard, Belchertown, MA; CFF= Clarkdale Fruit Farm, Deerfield, MA; ALC = UMass Ag. Learning Center, Amherst, MA; RGO = Roger's Orchard, Southington, CT.
[b]Ag-R = Ag-Radar; TT = trap trees, potted trees placed in orchards for each potential infection period.

Figure 1.1. ‘McIntosh’ apple, phenophase “green-tip”.

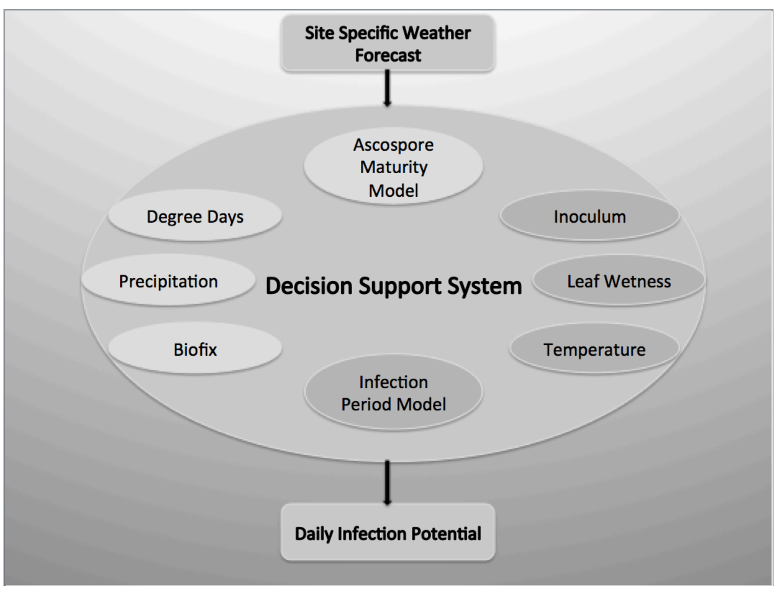

Figure 1.2. Confluence of climate and organismal development data to inform development of forecast models contained within DSS aiding in optimized disease management strategies.

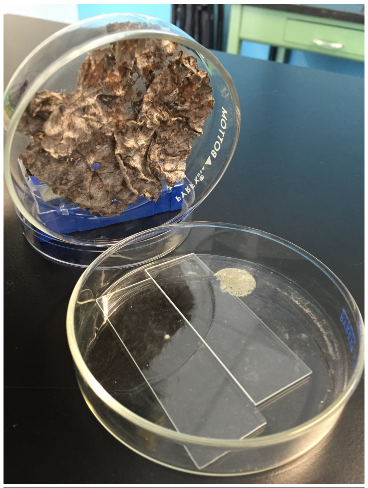

Figure 1.3. Petri plate assay method of monitoring ascospore development in season by counting spores ejected onto glass slides.

CHAPTER 2

DETERMINING CHANGES IN CLIMATE PARAMETERS POTENTIALLY AFFECTING DEVELOPMENT AND IINFECTION IN THE FUNGUS *VENTURIA INAEQUALIS*

2.1 Abstract

Weather affects all aspects of life on Earth, including agricultural pests. Seasonal development of these pests can be predicted using weather-based forecast models. However, many of these models are empirical, and changes in weather patterns caused by climate change can decrease their accuracy. The models may be improved by assessing regional climate changes; specifically their impact on weather parameters that impact pest forecast models. Periods of dry weather, characterized by consecutive days without precipitation, or precipitation-free periods (PFPs), and temperature can impact fungal pathogen development. PFPs and degree-day base 0° C ($DD_{0°C}$) are important factors in models used to forecast development and infection potential of the apple scab pathogen, *Venturia inaequalis*. This study assessed annual changes in PFPs and accumulations of $DD_{0°C}$ at six locations across New England from 1950 to 2017. Across sites there is no significant change in the number of four-day PFPs from 1950-2017; however, the CP decreased significantly ($p=0.013$). Seven-day PFPs did not change significantly across all sites from 1950-2017, but increased significantly during the RP ($p=0.019$). Annual accumulated $DD_{0°C}$ increased significantly across all sites from 1950-2017 ($p=0.007$). At Burlington, VT, total $DD_{0°C}$ increased significantly in the CP ($p<0.0001$). These changes in $DD_{0°C}$ accumulations have the potential to impact accuracy of ascospore maturity models that estimate infection risk during primary apple scab season.

2.2 Introduction

Knowledge of global climate change has spread, with some notable exceptions, as have its consequences. Climate affects plant growth and distribution and has the ability to impact plant pathogen development and disease (Coakley et al., 1999). The effect of greenhouse gasses on plant pathogens and the diseases they cause is well studied (Evans et al., 2008; Garrett et al., 2006; Juroszek & von Tiedemann, 2011; Luck et al., 2011; Wolfe et al., 2018). Impacts of rising CO_2 on plants may be direct, while indirectly impacting disease. For example, growing concentrations of atmospheric CO_2 may cause an increase in leaf size as well as density, raising humidity in plant canopies and exacerbating the occurrence of disease therein (Manning & von Tiedemann, 1995). Increased atmospheric CO_2 also leads to decreased decay of overwintering leaf litter which often contains pathogen inoculum (Ball, 1997).

Climate change also affects seasonal temperature and precipitation, factors critical to plant growth and plant disease epidemics (Pautasso et al., 2012). Warmer, earlier springs with extreme weather patterns are leading to changes in what are considered typical growing seasons in the Northeast (Frumhoff et al., 2007; Kunkel et al., 2013; Wolfe et al., 2018). For example, Kunkle et al. (2013) showed that the frost-free periods for the northeastern United States have increased in length, leading to a longer growing season. In Empire apples, for example, bloom dates in New York now occur eight days earlier than they did in 1960 (Wolfe et al., 2005).

Climate change and resultant changes in weather patterns may also impact the major apple disease apple scab, caused by the fungus *Venturia inaequalis* (Cke) Wint. Using Gadoury and MacHardy's New Hampshire model (NH model) for *V. inaequalis* ascospore development (Gadoury & MacHardy, 1982), and The Computer Center for Agriculture Pest Forecasting data (CIPRA; https://bit.ly/2B7Flu3), Bourgeois et. al.

(2004) predicted that, as a result of climate change, in the future there will be a rise in the number of infection events during the primary apple scab season in Quebec, largely the result of significantly earlier inoculum availability and tree growth, while the end of primary inoculum availability stays constant.

The NH model was developed and tested in a region where environmental conditions during ascospore development seldom included extended dry periods. In the years after its development, research has shown that variable dry conditions can lead to delays in *V. inaequalis* ascospore development (James & Sutton, 1982a; Roubal & Nicot, 2016; Stensvand et al., 2005). Regions where growers and researchers implement the NH model may experience conditions drier or warmer than those seen in NH when the model was developed, requiring adjustments to the model (Rossi et al. 1999, Jankowski & Masny, 2014). If dry periods are not considered in ascospore maturity models, the models will estimate the end of primary apple scab infection potential before it actually occurs in the field (St-Arnaud & Neumann, 1990; Stensvand et al., 2005).

Efforts to incorporate dry periods into Decision Support Systems (DSSs) have not been completely successful (see Chapter 1). RIMpro, one of the four DSSs most used in apple pest management in the northeastern United States, accounts for the effect of extended dry periods on ascospore maturation by incorporating a PFP, "threshold for maturation". Within the framework of the program, this PFP threshold will arrest ascospore maturity when a specified number of days without rain have occurred. The default value is 3 days, though this may be changed by users (Bio Fruit 2013, https://www.rimpro.eu). Being able to adjust the PFP threshold is a means of increasing model accuracy in specific locations. However, this also requires a deeper understanding of the impact of PFPs on *V. inaequalis* ascospore development than the average DSS user typically possesses.

Ag-Radar, another DSS used in the Northeast, is programmed to slow the percent of seasonal ascospore availability when < 2.54mm of rain has fallen (G. Koehler, personal communication 29 Oct.2018). This DSS does not permit users to adjust dry weather parameters, making it less flexible than RIMpro. The fact that ascospore maturity models are empirical suggests that having the ability to adjust model parameters such as PFP thresholds in specific production regions can be valuable, but this requires research and testing to establish appropriate region-specific parameters (Rossi et al. 1999, Jankowski & Masny, 2014).

Ascospore maturation models for *V. inaequalis* may also need to be refined to reflect climate change impacts on winter conditions, because significant pseudothecial development occurs during winter months. This development would most impact the time when the first mature ascospores are available. To simplify the use of DSSs, most use the readily observed green-tip phenophase for a common apple cultivar in a region as the point when ascospores are first available. This biofix also starts the accumulation of degree-days driving further ascospore development. The green-tip biofix has generally been adequate (see Chapter 1). However, if winters are warmer, and precipitation changes from primarily snow to rain, or dry periods increase, the correlation between green-tip and the availability of the first mature *V. inaequalis* ascospores may be disrupted. The thermal optima for pseudothecial development in winter is lower than that required for ascigerous development in spring (Gadoury & MacHardy, 1982; James & Sutton, 1982a; Schwabe et al., 1989). It is important to understand how temperature and precipitation are shifting during pseudothecial initiation, pseudoparaphyses development and ascospore maturation and spore release and infection, to develop better models to predict ascospore development and infection risk.

Reducing uncertainty in disease forecast models is imperative, because the models are foundational tools in integrated pest management, enabling growers to reduce fungicide and pesticide use in general as demonstrated in the northeastern US (MacHardy, 2000; Prokopy, 2003). While many factors influence IPM adoption, if growers do not trust IPM methods, and the DSSs that employ them, they will not use IPM (Kaine & Bewsell, 2008). Since *V. inaequalis* ascospore maturation models in the Northeast often give poor estimates (Chapter 1), understanding why this is the case is the first step in improving them.

The goal of this research is to assess the impact that climate change is having on the number and length of dry periods in New England, since this environmental change can be expected to impact models that estimate development of *V. inaequalis* ascospore inoculum and the disease apple scab. Temperature, specifically degree-day accumulation, is at the core of maturation models, and may be changing as well. Because the original NH model was developed in 1979 and 1980 (Gadoury & MacHardy, 1982), and refined for forecasting in the early 1980s (MacHardy & Gadoury, 1985), it is relevant to compare weather data from the period up to the early 1980s to weather data from that time to the present.

2.3 Materials and Methods

2.3.1 Data source

Analyses were performed on precipitation free periods (PFP) and $DD_{0°C}$ accumulations in two different time periods. The first, 1950 to 1983, is the reference period (RP) related to the years prior to and including the development of the NH model. These years reflect the climate and weather conditions under which the ascospore maturity and infection period models were developed. The second time period, 1984 to 2017, represents the current period (CP).

Six locations throughout New England were selected as representative apple production sites which also had comprehensive data sets available for climatic variables of interest: Hartford, Connecticut (CTH); Belchertown, Massachusetts (MAB) (PFPs only); Amherst, Massachusetts (MAA); Lawrence, Massachusetts (MAL) ($DD_{0°C}$ only); Lake Massabesic, New Hampshire (NHM); Burlington, Vermont (VTB) and Belfast, Maine (MEB). In cases where there was more than one missing data point in a month, averages of two to three sites near the focus site were used to complete maximum or minimum temperature calculations. Precipitation data sets were complete and required no additional data points. Climate data was downloaded from NOAA Regional Climate Center's SC ACIS (http://scacis.rcc-acis.org). Daily $DD_{0°C}$ accumulation values were calculated using maximum and minimum temperatures which were then compressed into annual means using JMP Pro 14 (SAS Institute, Inc., Cary NC) by using the tabulate function and mean statistic. Annual PFP means were also calculated this way. Regression analysis was used to determine to what degree, if any, $DD_{0°C}$ accumulations and dry periods have changed. $DD_{0°C}$ accumulations were analyzed using daily maximum and minimum temperatures calculated using spreadsheet developed by Bill Klein of the Northwest Michigan Horticultural Research Center which can be downloaded for free from the Michigan State University Department of Horticulture, Northwest Michigan Horticulture Research Center, Reports and Resources webpage; (https://www.canr.msu.edu/nwmihort/nwmihort_resources_and_reports#NWSprdsheets). These calculations utilize the Baskerville-Emin method or averaging method with no upper limit cut-off (Baskerville & Emin 1969).

2.3.2 Precipitation-Free Periods

Occurrence of four-, seven-, and ten-day precipitation-free periods (PFPs) was assessed annually for the six New England locations previously described. Seven-day PFPs was selected based on the work done by Stensvand et al. (2005), which showed that if degree day accumulation was halted if seven consecutive days without precipitation occurred, and then restarted when more than 0.2 mm fell, the accuracy of the NH model was greatly improved. In dry years, using the seven-day PFP reduced the discrepancies between estimated and observed ascospore maturity by and average of eighteen days Stensvand et al. 2005). Four- and ten-day PFPs were selected to represent less and more extreme drought periods.

2.3.3 Temperature Changes

Annual $DD_{0°C}$ accumulations were also analyzed using data from six sites throughout New England (Fig. 1) using discreet periods, though these differed slightly from the seasonal periods described for the PFP analysis. The periods September through November (SON) and December through February (DJF) were the same, but the March through May (MAM) period was extended to include June (MAMJ). This adjustment better fits the developmental periods of the fungus (MacHardy, 1996). SON relates to the initiation of the sexual reproductive phase of *V. inaequalis*. DJF has traditionally been considered a quiescent period, however, this may not always be the case and can vary geographically (James & Sutton, 1982b). March through June (MAMJ) relates to the period during which ascospores develop, are released and cause infection. Daily temperature ($DD_{0°C}$) and precipitation data were then condensed down to seasonal accumulation and occurrence, respectively. These data were further compressed to annual means to analyze the shift in $DD_{0°C}$ accumulations for each location assessed. July and August were not considered, as these months do not relate directly to the reproduction of *V. inaequalis*.

2.4 Results

2.4.1 Precipitation-Free Periods

- Four-day PFPs (Table 1)

Regression analysis, using the hypothesis that the linear regression slope is significantly different from 0, no slope shows that the occurrence of four-day PFPs at six New England sites from 1950-2017 has not changed significantly. When periods across all sites were analyzed separately, there is a significant negative slope ($p=0.0125$), indicating that the number of four-day PFPs in the CP is decreasing. The significant positive slope ($p=0.0003$) in the RP at CTH shows an increase in four-day PFPs for this location and time period. Four-day PFPs decreased significantly at MAA in the CP ($p=0.01$). Four-day PFPs at MEB increased in the RP ($p=0.023$), the CP, however, decreased ($p<0.0001$). There were no significant changes in slope at any of the other site-year combinations.

- Seven-day PFPs (Table 1)

Regression analysis of the six sites shows seven-day PFP occurrence in the RP increased significantly ($p=0.019$). Seven-day PFPs at CTH in the RP increased significantly ($p=0.002$). MAB seven-day PFPs increased significantly for the 1950-2017 timescale ($p=0.01$) as well as in the RP ($p=0.006$). At all sites the slope increased significantly for seven-day PFPs at NHM during the 1950-2017 timescale ($p=0.0007$). Seven-day PFPs at MEB significantly decreased in the CP ($p=0.0008$).

- Ten-day PFPs (Table 1)

There was no significant change for any of the ten-day PFP sites and years analyzed, with the exception of MEB, where ten-day PFPs increased significantly in the 1950-2017 timescale ($p=0.014$).

2.4.2 Temperature Changes

$DD_{0°C}$ accumulations at all sites and years increasing increased significantly ($p=0.007$) (Fig. 2). RP did not change significantly, whereas, $DD_{0°C}$ accumulations in the CP increased significantly ($p=0.004$) (Fig. 3). In the 1950-2017 timescale, CTH $DD_{0°C}$ accumulations increased significantly ($p<0.0001$) (Fig. 4). There was no significant change in the RP. The $DD_{0°C}$ accumulations in the CP at CTH increased significantly ($p=0.0003$) (Fig.5). There was no significant change in $DD_{0°C}$ accumulations at MAA. MAL $DD_{0°C}$ accumulations in the1950-2017 timescale increased significantly ($p=0.013$). $DD_{0°C}$ accumulations at MAL in the RP decreased significantly ($p=0.037$) while the accumulations in the CP increased significantly ($p=0.0002$) (Fig.6). At NHM $DD_{0°C}$ accumulations did not change significantly In the 1950-2017 or RP timescales but increased in the CP ($p=0.046$). $DD_{0°C}$ accumulations in the1950-2017 and CP timescales at VTB increased significantly ($p<0.0001$; $p<0.0001$) (Fig.7; Fig.9). $DD_{0°C}$ accumulations in the RP did not change significantly (Fig.8). $DD_{0°C}$ accumulations in the 1950-2017 timescale at MEB increased significantly ($p=0.011$) (Fig. 10). There was no significant change in $DD_{0°C}$ accumulations at MEB for the RP or CP.

2.5 Discussion

Temperature and precipitation are critical factors in *Venturia inaequalis* ascospore maturation and development and infection potential. Changes in temperature and precipitation can influence key developmental phases of *V. inaequalis*. Models, like the NH model, that predict ascospore maturity and infection during primary scab season rely on heat unit accumulation and precipitation events to track the development and release of inoculum and subsequent host infection (Roubal & Nicot 2015).

The unpredictability of extreme weather events makes it difficult to determine consistent values in empirical models and validate their impact on model accuracy. We do, however know, that these events have a significant impact on crop and disease development (Porter et al. 2014). It is imperative to continue to monitor and understand the shifting nature of the climate in New England so that growers may be prepared for abnormally dry periods, climbing temperatures, and the impact that those have on pathogens affecting their crops. For example, a recent survey of apple growers from several New England locations identified weather-based crop management as the most critical challenge they currently face. More specifically, growers identified a need to better understand current, seasonal pest distribution and emergent pest issues as they each relate to changing weather patterns in order to reduce uncertainties in IPM-based models (Morton et al., 2017). Increasing $DD_{0°C}$ accumulations and shifting PFP trends across New England and at state levels, raise concerns for model accuracy and shifting in season management strategies. Increasing $DD_{0°C}$ accumulations are of particular interest to *V. inaequalis* models as this factor significantly impact the development of ascospores that cause infection to apple leaves and fruit. Furthermore, shifting climatic trends that vary by- and within- state may be contributing to inaccuracies in models. Kunkle et al. (2014) showed that the Northeast is experiencing, on average, ten more consecutive frost free days annually, extending the growing season as well as a yearly average of .39" more rain. The NH model has been shown to work well under the climatic conditions that were experienced in NH in those years observed (Gadoury & MacHardy 1982; MacHardy & Gadoury 1985). However, many studies have shown that when this model is deployed in a region that experiences different climatic trend, the model loses accuracy (James & Sutton, 1982a; Rossi et al. 1999; Roubal & Nicot, 2016; Stensvand et al., 2005). The Northeast is experiencing different climatic conditions than it used to. For example, Lawrence, MA regression analysis exhibited an increase in $DD_{0°C}$ accumulations (p=0.0002) (Fig. 6) in the CP, while the change in $DD_{0°C}$ accumulations at Amherst, MA in the CP was not significant (p=0.48) (Fig. 10). Regression of seven-day PFPs at Belchertown, MA increased during the RP (p=0.024) whereas no significant change is in evidence at Amherst, MA (Table 2). The climatic variability within a state is such that applying one empirical model to an area may not fit growers' management needs. Based on the variability seen across the Northeast, the same can be said for the region. Models were developed to aid in predicting the primary apple scab infection potential and occurrence of infection. Models, however, do not estimate ascospore maturity and infection potential accurately when extreme weather events occur that fall outside of the range of conditions under which the model was developed (see Chapter 1). Additionally, the inability to adequately incorporate extreme events into models makes it more difficult for growers to confidently rely on them when these unpredictable extreme weather events do occur. Future steps for this work should include similar analyses of other climatic variables relating to pathogen, insect, and host development such as insolation, relative humidity and other climatic variables. Similarly, upper thermal optima, the maximum temperature at which the fungus can effectively survive, occurrences need to be analyzed, in conjunction with dry periods to assess their impact on pseudothecial longevity contributing to a prolonged ascospore development period. It is clear that what was once considered a normal spring in a temperate region is no longer consistently experienced in New England and that these inconsistent weather events vary in their severity from location to location, making the need for more dynamic DSSs a critical part of the success of future IPM management programs.

Table 2.1. Site-period combinations with significant changes in four, seven and ten-day PFPs.

Four-Day Precipitation Free Periods				**Seven-Day Precipitation Free Periods**				**Ten-Day Precipitation Free Periods**			
Site[a]	**Regression Equations**	**Period**	**r^2**	**Site**	**Regression Equations**	**Period**	**r^2**	**Site**	**Regression Equations**	**Period**	**r^2**
All	Y= 49.34 – 0.022*Year	CP	0.18	**All**	Y= -25.58 + 0.01558*Year	RP	0.159	**All**	N/A	N/A	N/A
CTH	Y= -81.27 + 0.045*Year	RP	0.34	**CTH**	Y= -57.97 + 0.03067*Year	RP	0.254	**CTH**	Y= 25.72-0.01245*Year	CP	0.11
MAB	N/A[b]	N/A	N/A	**MAB**	Y= -20.7 = 0.01153*Year	All	0.095	**MAB**	N/A	N/A	N/A
MAA	Y = 68.34-0.03102*Year	CP	0.183	**MAB**	Y= -62.3 + 0.0327*Year	RP	0.210	**MAA**	N/A	N/A	N/A
NHM	N/A	N/A	N/A	**NHM**	N/A	N/A	N/A	**NHM**	Y = -10.23+0.005602*Year	All	0.07
VTB	N/A	N/A	N/A	**VTB**	N/A	N/A	N/A	**VTB**	N/A	N/A	N/A
MEB	Y = 132.3-0.06264*Year	CP	0.400	**MEB**	Y = 6983-0.0335*Year	CP	0.299	**MEB**	Y = -12.31+0.006776*Year	All	0.089

[a] CTH= Hartford, CT; MAB=Belchertown, MA; MAA=Amherst, MA; NHM=Lake Massabesic, NH; VTB=Burlington, VT; MEB=Belfast, ME.
[b] Non-significant site-year combinations represented with 'N/A' indicate no changes in the slope of the line.

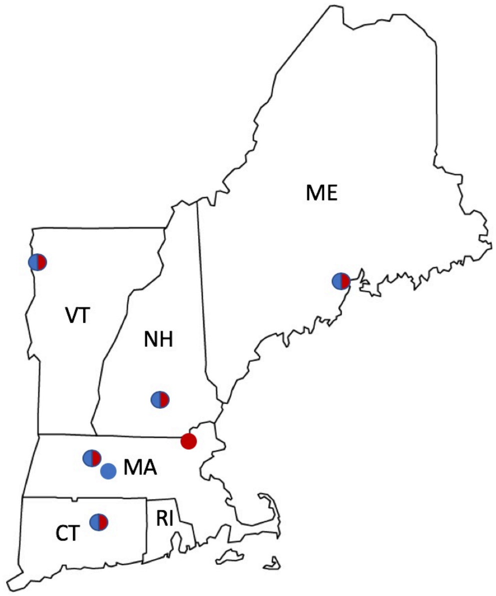

Fig. 2.1: Six New England locations used to analyze changes in four-, seven-, and ten-day precipitation free periods (PFPs) and changes in $DD_{0°C}$ accumulations. Bi-color dots indicate sites used for both analyses, solid blue for PFP only, and solid red for $DD_{0°C}$ only.

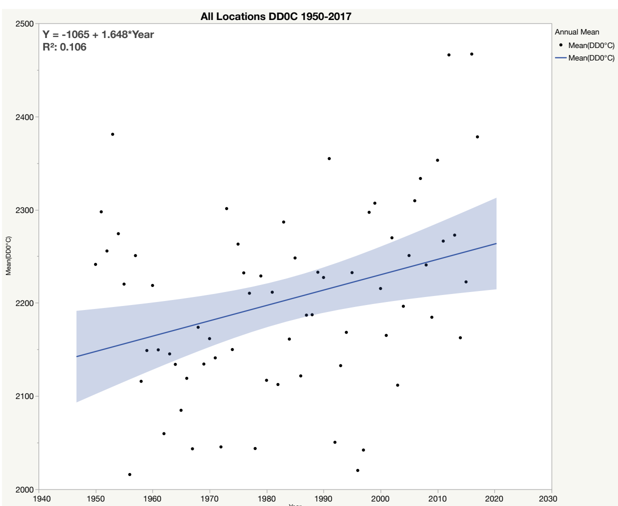

Fig. 2.2: Regression analysis showing a significant increase for all site and year $DD_{0°C}$ accumulations (r^2 0.106).

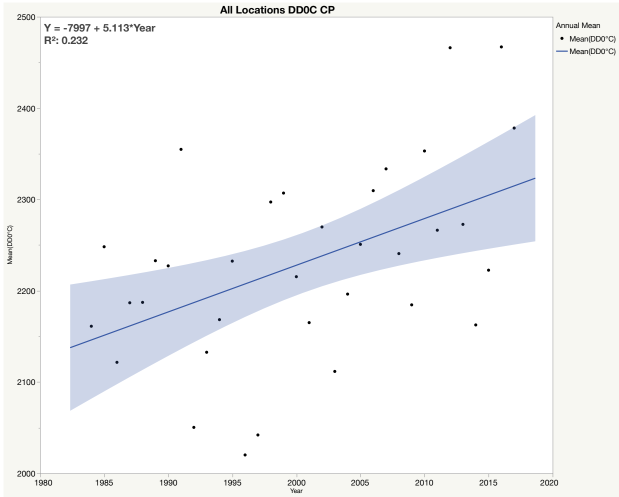

Fig. 2.3: Regression analysis of $DD_{0°C}$ accumulations showing significant increase for the CP at all sites (r^2 0.232).

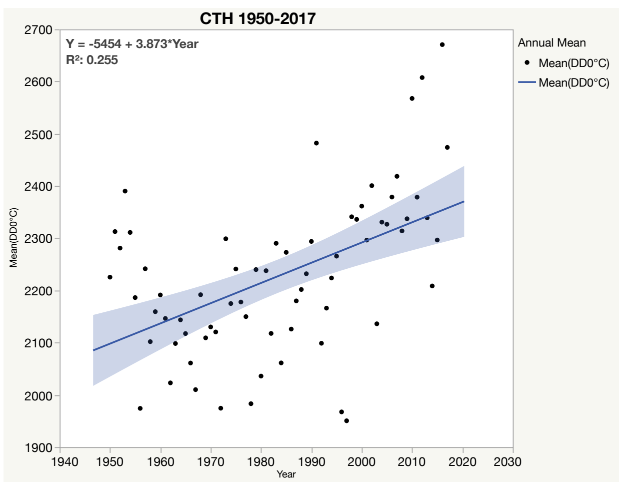

Fig. 2.4: Regression analysis of $DD_{0°C}$ accumulations showing a significant increase at Hartford, CT.

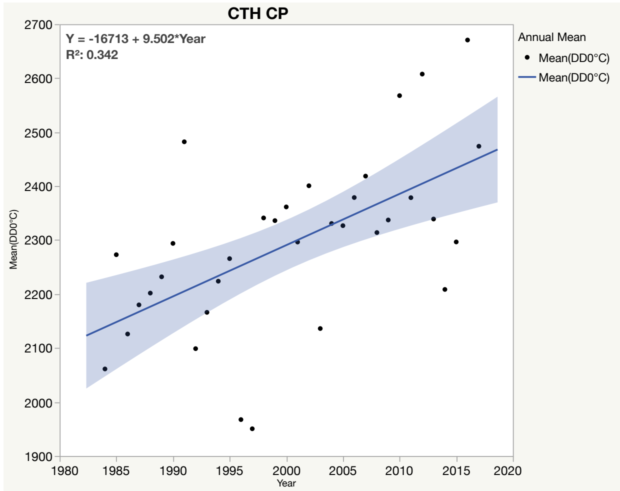

Fig. 2.5: Regression analysis of $DD_{0°C}$ accumulations showing a significant increase at Hartford, CT.

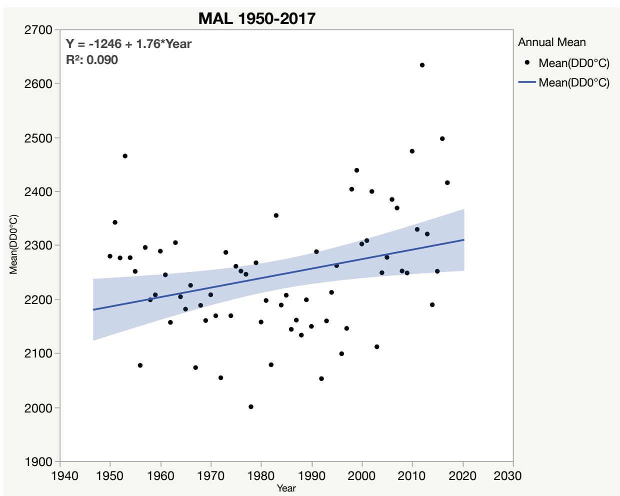

Fig. 2.6: Regression analysis of $DD_{0°C}$ accumulations at Lawrence, MA, showing a significant increase (r^2 0.09).

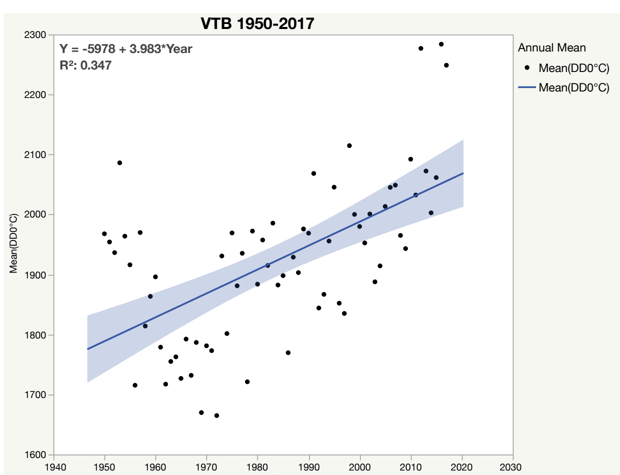

Fig. 2.7: Regression analysis of $DD_{0°C}$ accumulations showing a significant increase at Burlington, VT (r^2 0.347).

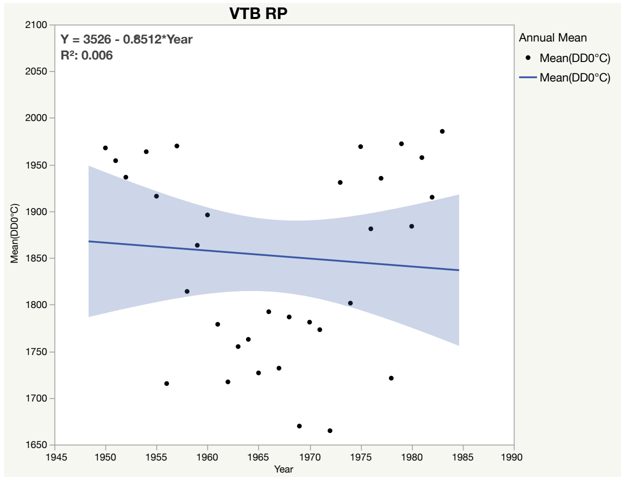

Fig. 2.8: Regression analysis of $DD_{0°C}$ accumulations at Burlington, VT shows no significant change (r^2 0.006).

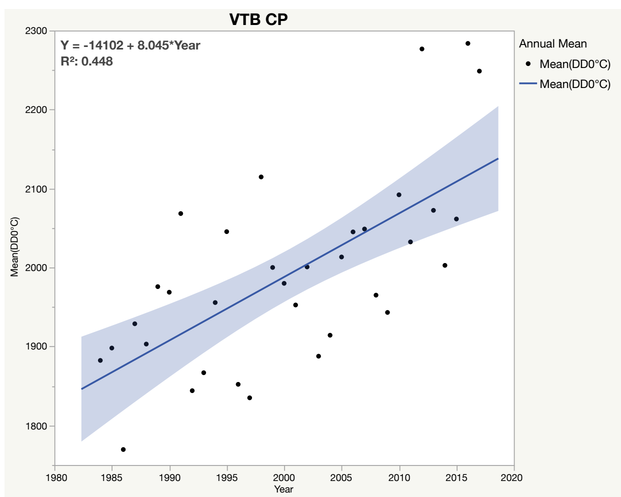

Fig. 2.9: Regression analysis of $DD_{0°C}$ accumulations at Burlington, VT shows a significant increase (r^2 0.448).

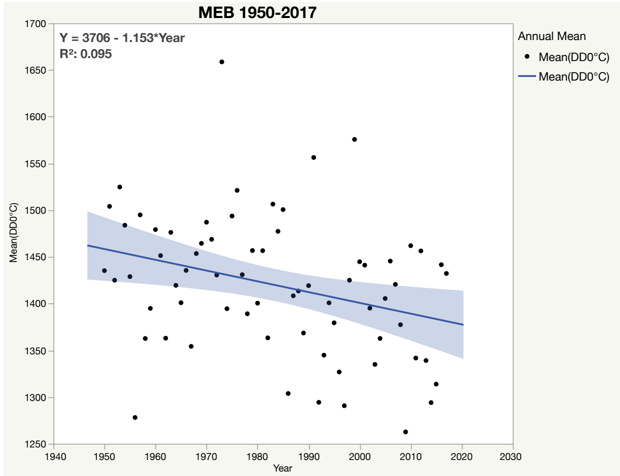

Fig. 2.10: Regression analysis of $DD_{0°C}$ accumulations at Belfast, ME shows a significant decrease (r^2 0.095).

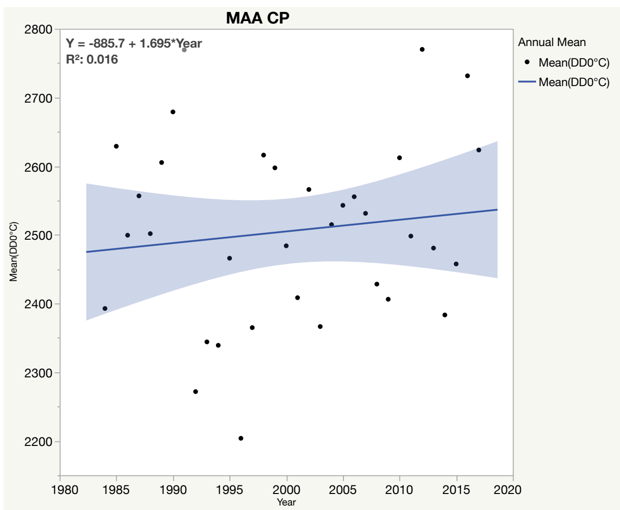

Fig. 2.11: Regression analysis of shows no significant change in $DD_{0°C}$ accumulations at Amherst, MA in the CP (r^2 0.016).

CHAPTER 3

UNDERGRADUATE STUDENT EXTENSION AND RESRAECH ENGAGEMENT INITIATVE: A CASE STUDY

3.1 Abstract

Engaging undergraduate students in agricultural education and research is a critical means of continuing to move forward in the field of sustainable food production. Getting students interested, and keeping their interest, will be necessary to train the next vanguard of Extension and academic professionals engaging in innovating research and education for the future of agriculture (O'Donoghue et. al. 2018). As the National Academy of Sciences (2009) states, the very nature of agriculture is changing, and land grant universities are responsible for leading the way to a better understanding of the future of sustainability.

A lesson plan appropriate for use in an undergraduate, introductory plant pathology class has been developed and accepted for publication in The American Phytopathological Society's *Plant Health Instructor.* This lesson plan will teach students key principals of integrated pest management, crop production, resistance avoidance, and the disease triangle. This plan will also introduce basic climate change principals such as increasing regional temperatures and precipitation variability and how these challenges may affect pathogenicity. All of these key principals will be taught using *Venturia inaequalis,* the fungal organism that causes the disease apple scab, as the model pathogen.

3.2 Introduction

This case study introduces undergraduate students to the management of apple scab (caused by *Venturia inaequalis*), a classic disease that drives most of the fungicide use in apples in the northeastern United States. It teaches the relevant biology of the pathosystem, and exposes students to disease forecast models and using them in a decision support system.

A young apple grower, Laura Sagar, has adopted new cultural control strategies, and weather-based disease models to estimate and forecast the risk of scab infections. Laura's father sprayed according to how long it had been since his last fungicide application, usually every five to seven days from early spring into early summer. Laura, and her customers, wants to keep fungicide use at a minimum. Cultural controls and disease forecasts offer a way to reduce disease pressure and fungicide sprays while maintaining crop quality. The combination works well in Laura's first years managing the orchard, but then a devastating scab epidemic nearly destroys her crop, causing her to ask whether she should return to her father's calendar-based approach to scab management.

The apple scab management failure case asks students to learn the biology of apple scab, and to understand how the epidemiology of scab has been used to design integrated pest management (IPM) approaches for scab. By determining how the scab epidemic in Laura Sagar's orchard happened, students will learn about effective, ecologically based tactics for managing plant diseases, such as inoculum reduction, monitoring weather and using disease risk forecasts.

3.2.1 Objectives

The goal of this case study is to teach students modern methods for plant disease management by asking them to determine the reasons behind the failure of a disease management program. Students will learn the basics of two empirical disease forecast models, the importance of weather in such forecasts, and how they are used in an IPM program. The case study will also show that IPM involves more than fungicide management.

After completing this case study, the student will:

- Recognize apple scab symptoms.
- Understand the apple scab disease cycle and the role played by weather in scab infections.
- Understand the importance of primary inoculum and managing primary infections in a polycyclic disease.
- Be able to weigh the advantages and limitations of timing fungicide applications using weather forecasts and related models.

3.2.2 Cast of Characters

Laura Sagar – Apple orchard owner/manager. She inherited the orchard from her family.

Noah Elma - Extension fruit specialist in western Massachusetts, working to aid fruit growers in the use of IPM, particularly cultural controls and disease forecasting models, in order to reduce pesticide use while maintaining crop quality.

Jennifer Shea – Plant pathologist.

3.3 The Case

Laura Sagar had been learning the ropes of managing her families' apple orchard from her father from the time she was a small child through high school and college, and since graduating she had been anxiously waiting to run it herself. In 2009, when her father Jerry reached his 65th birthday, he sold the Sagar Family Orchard to her, and left the Massachusetts farm for Aruba. Laura had the independence she'd long wanted, but for the first time felt the full worry and stress of making her living from the farm.

High among those worries was managing apple diseases. Apples get many diseases, most caused by fungi; the most important of these is apple scab (Figure 1). The apple varieties McIntosh and Cortland, preferred by Laura's customers, are especially susceptible to scab. In Massachusetts, a normal spring is rainy and cool, providing the perfect conditions for apple scab infections. If apple growers fail to manage the disease, scab can be devastating, destroying most or all of a crop. Laura's father, like many conservative, older growers had managed scab using frequent applications of fungicides, materials that kill or otherwise inhibit the growth the pathogen that causes scab, the fungus *Venturia inaequalis.* To do this, he used a huge airblast sprayer (Figure 2), a machine that sprayed a mist of pesticides into a fan the size of a small plane's propeller, driving clouds of fungicide solution onto the trees. His strategy was simple: keep the trees covered with fungicide from the time the first green leaflets emerged in spring until after trees had bloomed and fruit had begun to form. Usually, he had to apply fungicide sprays targeting scab about ten times each year, sometimes more. Even then, occasionally there would be apples with scab at harvest, and every scabby apple was considered a worthless apple.

Laura didn't really enjoy driving the tractor up and down the rows, often in the night, with the roaring sprayer at her back, but there was really no alternative if she wanted to stop scab and other disease and insect problems. She knew her customers, and consumers in general, worried about pesticides on fruit. So when Laura heard about a way to cut sprays without increasing the risk of pest damage, it caught her interest. The approach, Integrated Pest Management (IPM), decreased reliance on pesticides, and incorporated other tactics to manage diseases and insects effectively. It meant spending more time every day gathering information about the insects and diseases, but she hoped it would reduce the time and money she spent spraying. Over the three years she had been using IPM, it had.

IPM programs require that a grower understand the biology of the pest to be managed, in this case, the fungus *Venturia inaequalis* (Figure 3). Laura had learned that scab epidemics happen in two phases, primary infections and secondary infections. Primary infections start an epidemic, and are caused by the apple scab fungus, which survives winter in old leaves on the orchard floor (Figure 4). Usually, the first spores are ready to be released just as apple trees emerge from dormancy in the spring. This is when the trees are pushing out new green leaves (Figure 5). Wet weather is critical to the development and release of fungal spores, called ascospores (Figure 6), which float into the air. Spores that land on the new apple leaves germinate, producing a small tube, a hypha (Figure 7), and if the leaf stays wet for long enough, the tube penetrates the leaf's cuticle. Once inside, the fungus continues to grow along the leaf surface, between the cuticle and epidermis. Eventually the fungus shoves its way back out through the cuticle, forming a fuzzy mat containing tens of thousands of new spores (Figure 8). These spores, called conidia, can each start new, secondary, infections, which in turn can produce another generation of conidia, and in a few weeks, a scab epidemic can explode.

The amount of time it takes a scab infection to go from penetrating a leaf to producing conidia depends on temperature. At relatively warm temperatures, for example 65 to 75° F, it takes nine days; when it's cold, say 45 to 55°F, it takes 17 days. Before the new scab spots become visible, growers can't see the fungus growing. It's invisible. They have to depend on understanding the conditions that lead to infection in order to make good management decisions.

Laura knew the key to apple scab IPM is preventing primary infections. "If primary scab infections are prevented, or reduced," the IPM specialist had explained, "there is no need to spray for secondary scab infections. There's no inoculum."

The apple IPM program involved collecting a lot of information. For apple scab, Laura started by estimating how much inoculum would be in the orchard at the beginning of the season. This meant going through the orchard after harvest, before leaves drop, in the fall and systematically counting the number of infected leaves on a sample of trees after harvest. It took time when she was already quite busy selling apples, but it gave her a clear indication of the relative risk of scab for the following season. Ascospores from *V. inaequalis* usually don't travel very far, about 100 feet or so. That means most, if not all, of the inoculum comes from leaves that fall within an orchard. If there are very few scab-infected leaves at harvest, the risk of scab next year is low. Better yet, the risk of scab infections when leaves first emerged in spring can be so low that there's no need to spray as soon as the first green apple tissue emerges, so the first fungicide application, or even two, that her father had always made could be skipped. On the other hand, if Laura found enough scab-infected leaves, she knew she should start scab management as soon as the first leaves emerged.

The next part of her IPM program involved sanitation, which meant destroying as much primary inoculum in the orchard as she could. To do this, Laura would spray the trees with a common nitrogen compound, urea, just before the leaves fell. If she was too busy, she could spray urea on the fallen leaves, either in the fall or the spring. After that, she would use a kind of brush and grass mowing machine, a flail chopper, to grind the leaves to bits. The nitrogen in the urea fed bacteria and other microbes that would quickly decay the leaves, while chopping further promoted leaf decay, and disrupted fungal growth. It was a kind of insurance against inoculum that might be in the old leaves.

In spring, when the apples began to break buds, Laura kept careful track of the weather. Weather, temperature and moisture specifically, are an important part of any pest's development. She had purchased an electronic weather station that fed data into her office computer, then over the Internet to a computer at a university, where it was run through different pest forecasting models. Laura could use a web app to look at the output. This app told her whether an infection had occurred, and using data from weather forecasts, whether an infection is likely to be coming. It all happened virtually instantaneously. Using the information, Laura could decide whether she needed to spray. Basically, the app told her when the scab fungus was producing ascospores and when it stopped making them (Figure 9), and if rainy weather may be sufficient to lead to a scab infection (Figure 10). Laura didn't understand the details of the models in the app, but they had worked well for her so far.

She knew that whether or not ascospores cause primary infections depends on wet leaves, so growers need to keep a close eye on "wetting periods" and their associated temperatures. Not all wetting periods cause infection. Wet periods that can cause infections are known as infection periods, or Mills Periods, after a scientist who discovered that the time needed for infection varies with temperature. At cold temperatures, near freezing, it takes as long as two days for the scab spores to germinate and infect wet leaves. At warmer temperatures, around 60 to 75 °F, it takes only nine hours of leaf wetting for the fungus to infect. If a weather forecast predicted infection conditions, it would show up on the app, and Laura would apply fungicide protection if she needed to. Alternatively, with some fungicides, it is possible to spray after an infection has started, and Laura would use these post-infection sprays if needed. In any case, she could use the app to overcome the invisibility problem with early scab infections, so she could tell here whether or not an infection had occurred.

In the fall of 2014, Laura found herself too busy with sales to spend time evaluating the amount of scab in her orchard. Recent springs had been very dry, and the orchard hadn't had any significant scab for a couple of years. The search for one or two scab leaves in the orchard had gotten monotonous. She decided it would be okay to skip the inoculum monitoring that fall. And again, because she was busy, she decided to put off her sanitation treatment until spring.

The spring of 2015 began wet and muddy as usual for the Northeast. Laura was a little nervous about not being able to get into the orchard to spray urea and chop leaves before the apple buds opened, but since she hadn't had scab problems for several years, she didn't think it would cause problems.

Buds swelled, burst open and started to produce flower buds; she watched the weather and the scab forecasts on her computer. As had become her practice in her low-inoculum orchard, she didn't spray for the first infection period.

Then, abruptly, the rain disappeared, and a couple of days of hot dry weather dried the ground. A few days later, she put on a fungicide for scab when a cold front generated a couple of days of rain, enough for an infection according to the app forecast. After that, it stayed warm and dry for a couple of weeks, oddly so for a region that usually spent spring more or less soggy. When the petals began to fall from the apple flowers and the fruit begin to form, the rain returned for a week. Laura saw the rain coming using the app, and sprayed a fungicide to protect against infection. Normally, this would be the last primary scab spray Laura would need to apply that season. According to the app, ascospores had all matured. Primary scab was over. This year looked more or less normal, if a little dry. The temperature-based model and her father's conventional wisdom suggested that inoculum had all been released a week after petal fall. Her fungicide protection should have dealt with the last infection. Laura heaved a mental sigh, deciding scab sprays were done. The bloom on her apples had been heavy, setting a good crop, and her harvest promised to be excellent.

Several weeks after the end of primary scab season, Laura was out checking for insect damage in the orchard, and was horrified to discover velvety, olive-colored spots on many of the leaves. This meant at least three, four or even more fungicide applications would be needed to try to stop the epidemic from infecting fruit. Otherwise, come harvest, most of her apples would be unmarketable, and she would have a hard time making ends meet. Laura was angry and frustrated. She had followed the IPM strategy that had been working well for her for several years, yet the disease had hit her anyway. What had gone wrong???

3.3.1 Questions

1. What is the most critical period in the apple growing season for scab management?

2. What are two distinct stages in an apple scab epidemic, and what types of fungal spores are associated with each?

3. What two environmental factors are critical in apple scab infections? Which one drives the development of primary inoculum? Which is/are important in an individual infection of apple tissue?

3.4 Disease Management

In a bit of a panic, Laura called her local Extension tree fruit specialist, Noah Elma, whom she had worked with to develop the IPM plan for her orchard. Together they installed the weather station. He also connected Laura with the university.

First, Noah grilled Laura on how much fungicide she applied in her sprays, whether she had calibrated her sprayer to apply correct amounts, and whether wind might have been blowing hard enough to cause the fungicide to drift away from the apple trees where it was needed. Laura kept detailed records of all her pesticide applications. These records include information like date and time of application, what materials and rates she used and a brief description of what the weather looked like for any given day. Based on Laura's records, it didn't appear as though wind or the amount of fungicide used were at fault.

Then they poured over the records from Laura's weather station. There had been four infection periods during the time that models predicted ascospores were available to cause infections, two early and two late in the primary scab season. The pair then checked Laura’s fungicide spray records from that spring to verify that the orchard had been treated at the right times for each of the infection events.

“You didn’t put anything on for the first infection?” asked Noah.

“No. It was muddy in the orchard. And for the last few years, I’ve skipped the first one. No problem.”

“Let’s go look,” said Noah.

They went back to Laura’s orchard and pushed into the leaves on the trees to see exactly what the pattern of infection was. Right away, Noah saw that most infections were on leaves that had emerged relatively recently. In fact, some infections seemed to have happened after all inoculum should have been spent. It didn’t make sense, the model clearly showed primary scab inoculum depleted at least 10 days before some leaves had emerged and been hit with scab. Laura’s spray records indicated she had gotten a protective cover on in advance of each of the other primary infection events.

“Except for the first one”, observed Noah.

“I explained that. Shouldn’t have been a problem”, snapped Laura.

“What did your fall scab survey show?”

“Actually, I had to skip it last fall.”

“I’m thinking maybe that was a bad idea. You made a lot of assumptions.”

Noah’s idea was that Laura had been hit by a combination of failing to monitor and bad luck. Laura’s orchard had likely suffered a little more scab than usual the previous year, and she hadn’t noticed because she hadn’t done her usual careful fall evaluation. Normally, her sanitation program would have greatly reduced or eliminated most inoculum. But it hadn't happened. But, where had the late season infections come from?

"Two possibilities," said Noah. You got some early infections in that first infection period, and it didn't explode to the point you saw it until now. The other is a little strange. I've heard Jenn Shea say that the model for predicting the end of primary season may not be that accurate in dry years."

Noah gave Jennifer Shea, a plant pathologist at the University of Massachusetts, a call. After hearing Laura's tale of woe, Jennifer agreed. She had observed the actual growth and release of ascospores in order to check the model estimates. This year, the most commonly used model, the one Laura followed, had indicated primary season was done nearly two weeks before Jenn had stopped seeing mature spores. In that time, there had been a significant infection period. She had also fielded calls from other growers; Laura was not the only one with an apple scab outbreak, though hers' was noticeably more severe.

The high number of infections in her orchard still surprised Laura. There shouldn't have been much inoculum left at the end of ascospore development. Noah reminded her of the possibility of an infection very early in the season.

"The earlier scab starts, the worse the epidemic Laura. You know that."

With more inoculum than usual in her orchard, any mistake, would lead to more scab than usual. Some of the mistakes had been Laura's. The fall assessment would've warned her of a potential problem. Sanitation would have helped reduce the impact, had she done it. If she had checked her "hot spots", the places where scab was most likely to hit, during the spring, she might have seen symptoms earlier and been better able to stop them.

“Yeah, I got kind of complacent. But what about the end of scab season thing?”

“I don’t know. Hopefully Jenn will fix that soon. Meanwhile, with these climate change extremes, dry then wet, I’d play it conservative. The decision support recommendations are a useful guide, but they aren’t absolute reality.”

It made sense, though it didn’t make Laura feel any better. It would be a rough year. She launched a fungicide program to kill off the scab infections and protect fruit from new, secondary infections. Ultimately, she sprayed twice as much as she normally had, and still suffered some loss from scab at harvest. It had cost her much more than usual. From then on, monitoring and the other IPM tactics she used for scab became a top priority: evaluating inoculum in the fall, spraying and chopped leaves in the fall, and interpreting the app recommendations more conservatively.

3.4.1 Questions

1. What are the three key factors that could have contributed to the scab epidemic in Laura’s orchard?

2. How does evaluating inoculum in the fall contribute to an apple scab management program?

3. Why is it important to know when all primary inoculum is spent? How might Laura use this information to inform scab management decisions?

4. Why is it important for Laura to keep detailed records of her management decisions?

Figure 3.1. New apple scab infections on an apple leaf early in the season (top left); older leaf infections that have darkened (top right); lesions on young fruit (bottom right) and fruit at harvest (bottom left). (D. R. Cooley, Univ. of Mass.)

Figure 3.2. Airblast sprayer applying pesticides in an applied research apple orchard. (Jon Clements, UMass Extension)

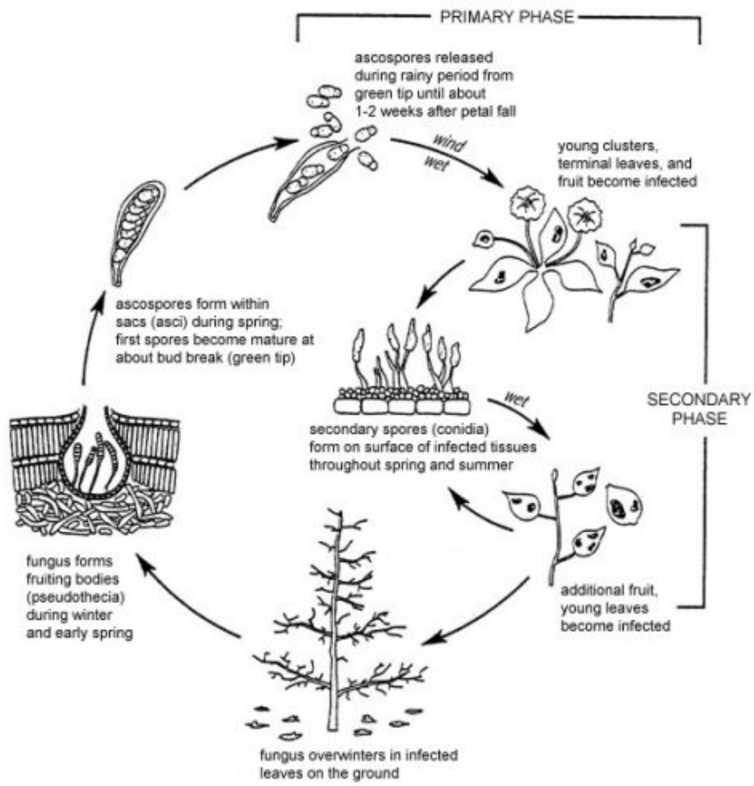

Figure 3.3. Apple scab disease cycle. (American Phytopathological Society http://www.apsnet.org/edcenter/intropp/lessons/fungi/ascomycetes/Article%20Images/AppleScabdiscycle.jpg)

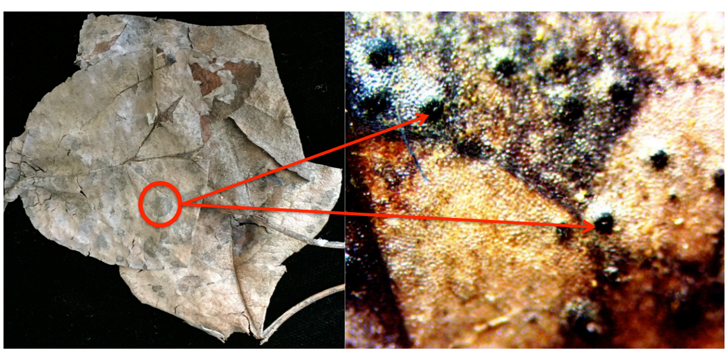

Figure 3.4. Right: apple leaf from an orchard floor showing scab infections from the previous growing season (dark areas). Left: magnified scab lesion in a leaf showing several fruiting bodies (round, dark objects), called pseudothecia, with one pseudothecium circled. (E.W. Garofalo)

Figure 3.5. The green tip growth stage on apple, when buds break and begin to form the first leaves. (Jon Clements, UMass Extension)

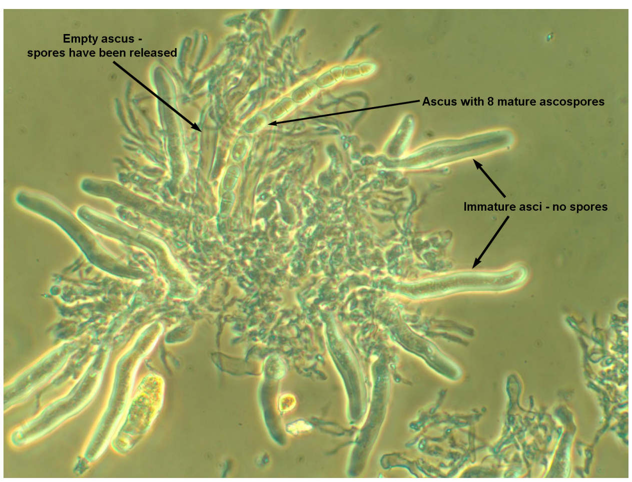

Figure 3.6. *Venturia inaequalis* pseudothecium magnified under a microscope (400X) in a prepared “squash mount” showing the three important stages of ascospore maturation: immature asci with no spores or immature spores; a mature ascus with mature ascospores; and an empty ascus, which has discharged spores. (D. R. Cooley, Univ. of Mass.)

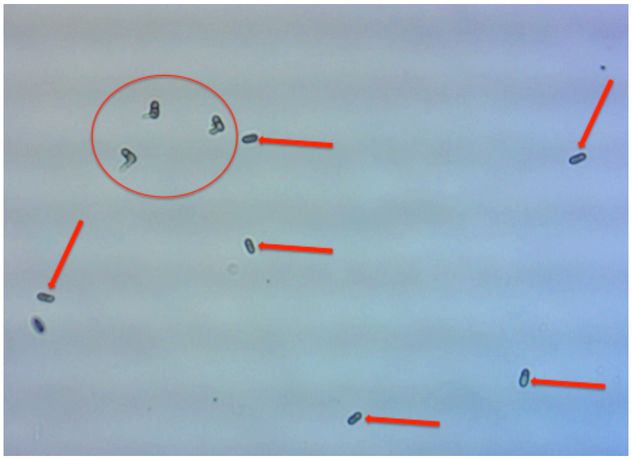

Figure 3.7. *Venturia inaequalis* ascospores ungerminated (red arrows) and germinating (red circle), producing hyphae, which can penetrate apple tissue. (E.W. Garofalo)

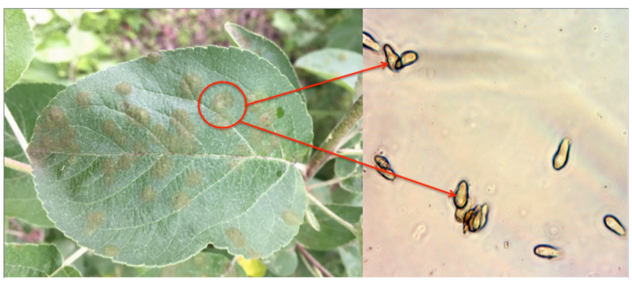

Figure 3.8. New scab lesions on a leaf (left); conidia that have developed as a result of infection (right). (E.W. Garofalo)

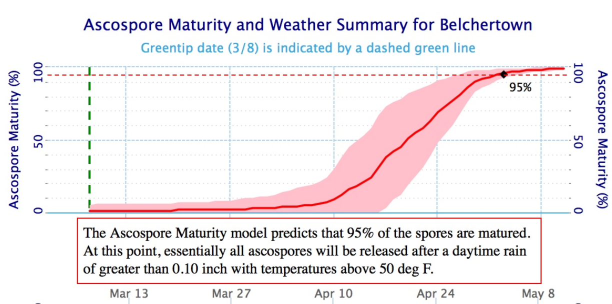

Figure 3.9. Output from a decision support system (NEWA) for apple scab indicating estimated ascospore maturation. At this site during this year, accumulated ascospore maturity reached 95% on May 2 and 100% on May 15.

Infection Events Summary

	Past	Past	Current	Ensuing 5 Days				
Date	3/29	3/30	3/31	4/1	4/2	4/3	4/4	4/5
Infection Events	No	No	No	Combined	Combined	Yes	No	No
Days to Symptoms	-	-	-	-	-	17	-	-
Average Temp (F) for wet hours	-	-	-	61	42	33	24	22
Leaf Wetness (hours)	0	0	0	15	12	12	9	4
Hours ≥90% RH	0	0	0	7	6	6	12	0
Rain Amount	0.00	0.00	0.00	0.22	0.13	0.05	0.00	0.19

Download Time: 4/6/2016 23:00

Infection events, shown in red above, are based on the Revised Mills Table and are calculated beginning with 0.01 inch of rain. The word "Combined" means the wetting event on this day is being combined with another wetting event using the following rule: two successive wetting periods, the first started by rain, should be considered a single, uninterrupted wet period if the intervening dry period is less than 24 hours. **When an infection event is in the 5-day forecast, the actual weather data logged may or may not translate into an actual infection event. Therefore, the table output may change once actual weather data are logged.**

Figure 3.10. Output from a decision support system (NEWA) for apple scab indicating infection events from Mar. 29 to 30, and forecast to Apr. 5, indicating a high risk of infection on Apr. 1 to 3, with relevant related data and forecasts.

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